

LOCALIZATION OF γ -GLUTAMYL TRANSPEPTIDASE IN LYMPHOID CELLS

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1. Introduction

γ -Glutamyl transpeptidase catalyzes the initial step in the breakdown of glutathione. A proposed role for the enzyme in transport processes [1,2] is consistent with its localization in the plasma membranes of a variety of epithelial cells [3,4]. The enzyme is also present in human and rat lymphoid cells and the activity can be increased by treatment of the cells with mitogenic agents [5]. The transpeptidase activity of human lymphoblastic lines varies considerably; cell lines from patients with lymphoproliferative diseases exhibit activities significantly lower than lines obtained from normal subjects, whereas the highest activity was observed in a line derived from a multiple myeloma patient [5].

Ultrastructural localization of the transpeptidase activity, results of which are reported here, show that the lymphoid cell plasma membrane is the primary site of enzyme activity. A largely uniform distribution of enzyme on the cell-surface is indicated from experiments with ferritin-antibody conjugates. Although short-term incubation of cells in cytochemical medium results in staining of the plasma membrane only, longer incubation reveals activity in the membranes of the endoplasmic reticulum and the Golgi. This intracellular activity may be associated with intermediate stages in the processing of the enzyme en route to its insertion in the plasma membrane and may also function in the turnover of intracellular glutathione. The similarity of the lymphoid cell enzyme to the purified kidney enzyme is evident from antibody inhibition studies and gel electrophoresis in sodium dodecyl sulfate (SDS).

2. Materials and methods

L- γ -Glutamyl-*p*-nitroanilide, glycylglycine, neuraminidase and galactose oxidase were purchased from Sigma. L- γ -Glutamyl-(4-methoxy)-2-naphthylamide, 4-aminophthalhydrazide, osmium tetroxide, and ferritin were obtained from Polysciences. NaB^3H_4 (100 mCi/mmol) was from New England Nuclear. Antibodies against human kidney γ -glutamyl transpeptidase (isolated as in [6]) were prepared in rabbits and the γ -globulin fraction was purified [7]. Conjugation of the IgG fraction with ferritin was done as in [8].

Human lymphoid cell lines, RPMI 1788 (from a normal subject) and RPMI 8226 (from a patient with multiple myeloma), both exhibiting B-cell characteristics, were obtained and maintained as in [5]. The transpeptidase activities of these lines were 990 and 11 000 units/mg, respectively (nmol *p*-nitroaniline formed $\cdot \text{h}^{-1} \cdot \text{mg protein}^{-1}$ when assayed as in [5]).

Labeling of the cell-surface glycoproteins and isolation of labeled transpeptidase were done as follows: RPMI 8226 cells ($\sim 2 \times 10^7$) were washed and suspended in 1 ml 0.01 M phosphate buffer (pH 7.4) containing 0.15 M NaCl (phosphate-NaCl). Treatment with neuraminidase and galactose oxidase followed by labeling with NaB^3H_4 was done essentially as in [9]. In controls, galactose oxidase was omitted. The cells were then centrifuged, washed several times with phosphate-NaCl, finally suspended in 1 ml buffer containing 0.2% Lubrol WX, and homogenized in a glass-teflon homogenizer. The homogenate was centrifuged at $18\,000 \times g$ for 60 min; the supernatant contained all of the cellular transpeptidase activity. Purified human kidney transpeptidase ($4 \mu\text{g/ml}$) was added to the extract and the enzyme was precipitated with anti-human kidney transpeptidase IgG. The immunoprecipitates were collected by centrifugation,

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washed thoroughly with phosphate-NaCl buffer containing Lubrol WX and finally dissolved in 0.1 ml 2% SDS. The samples were subjected to electrophoresis in 8% polyacrylamide gels in the presence of 0.1% SDS, stained with Coomassie blue R-250, destained, sliced into 2 mm sections and the slices counted as in [9].

Ultrastructural localization of the transpeptidase activity in lymphoid cells was done as in [4,10]. Washed cells ($\sim 5 \times 10^7$) were mixed with 0.2 ml melted agar (2% in phosphate-NaCl) at 45°C. The solidified agar block was cut into small pieces and incubated at 25°C for 60 min in a medium containing γ -glutamyl-(4-methoxy)-2-naphthylamide, Gly-Gly and 4-aminophthalhydrazide. In controls, the transpeptidase activity was inhibited by the addition of L-serine and borate (5 mM each) to the incubation medium [11]. Osmication, dehydration, and embedding in Araldite was done as in [4]. Ultrathin sections were cut on a LKB ultramicrotome and observed in a JEM-100B electron microscope.

Lymphoid cells were treated with the ferritin conjugate of anti-human kidney transpeptidase IgG as in [4]. Cells were prefixed with 1% formaldehyde in phosphate-NaCl for 30 min at 4°C. After washing, the cells were incubated with an excess of ferritin-IgG conjugate for 2 h at 4°C. In controls, the prefixed cells were exposed to unconjugated anti-IgG prior to the treatment with ferritin-IgG. Excess conjugate was removed by washing after which the cells were fixed in 2.5% glutaraldehyde in phosphate-NaCl and processed for electron microscopy [4].

3. Results

Treatment of intact cells with anti-human kidney transpeptidase serum resulted in $\sim 70\%$ inhibition of the cell transpeptidase activity (fig.1, curve 1). The activity of the detergent extract of the cells was sim-

ilarly inhibited and overnight incubation with the anti-serum resulted in complete precipitation of the solubilized enzyme (fig.1, curve 2). Similar inhibition and precipitin curves were obtained when an equivalent

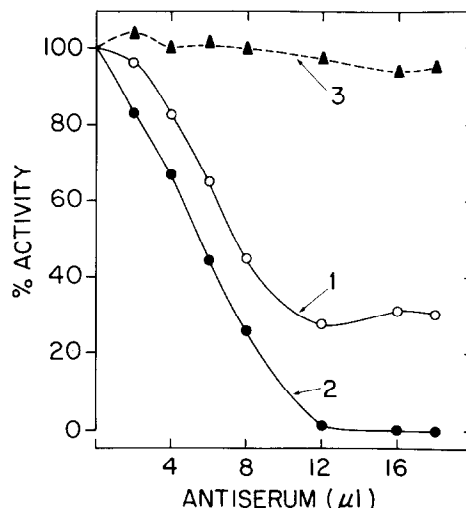


Fig.1. Effect of human kidney γ -glutamyl transpeptidase antiserum (rabbit) on the transpeptidase activity of lymphoid cells. (1) Human lymphoid cells ($\sim 10^7$ RPMI 8226 cells in 1 ml phosphate-NaCl; 11 000 units enzyme) were treated with the antiserum (diluted 1×25) for 1 h at 25°C. Aliquots (0.2 ml) were taken for enzyme assays. (2) RPMI 8226 cells were homogenized with phosphate-NaCl containing 0.2% Lubrol WX (1 ml/ 10^7 cells) and the homogenate centrifuged at $18\,000 \times g$ for 60 min. The supernatant (1 ml), containing all of the cellular enzyme activity, was treated with the diluted antiserum as shown. After incubation at 4°C for 24 h, the solutions were centrifuged at $5000 \times g$ for 30 min and the supernatants assayed for residual activity. Similar inhibition and precipitin curves were obtained with an equivalent amount of purified human kidney transpeptidase. The activities of human peripheral blood lymphocytes and of cell line RPMI 1788 were also inhibited by the antiserum. (3) shows the absence of any effect of the antiserum on rat spleen cells which had been cultured 72 h after mitogenic stimulation with Con A as in [5] (1.2×10^7 cells/ml phosphate-NaCl; 3800 units enzyme activity).

Fig.2. Ultrastructural localization of γ -glutamyl transpeptidase in lymphoid cells. (A-E) Cytochemical localization of enzyme activity in human cell line, RPMI 8226. Note the thicker and darker appearance of the plasma membrane, PM, in (A) indicative of transpeptidase activity as compared to the appearance of PM in the control (C) in which the activity was inhibited. Activity was also seen in the membranes of the endoplasmic reticulum, ER (B) and the Golgi, G (D). (E) is the control for (D). (A-C) $\times 50\,000$; (D-E) $\times 30\,000$. (F-I) Treatment of human cell lines RPMI 8226 (F,G) and RPMI 1788 (H,I) with the ferritin conjugate of anti-transpeptidase IgG. Note the uniform binding of the conjugate to the outer surface of the plasma membrane of RPMI 8226 (F) and RPMI 1788 (H). (G,I) are the corresponding controls in which the cells were treated with the IgG prior to addition of the ferritin conjugate.

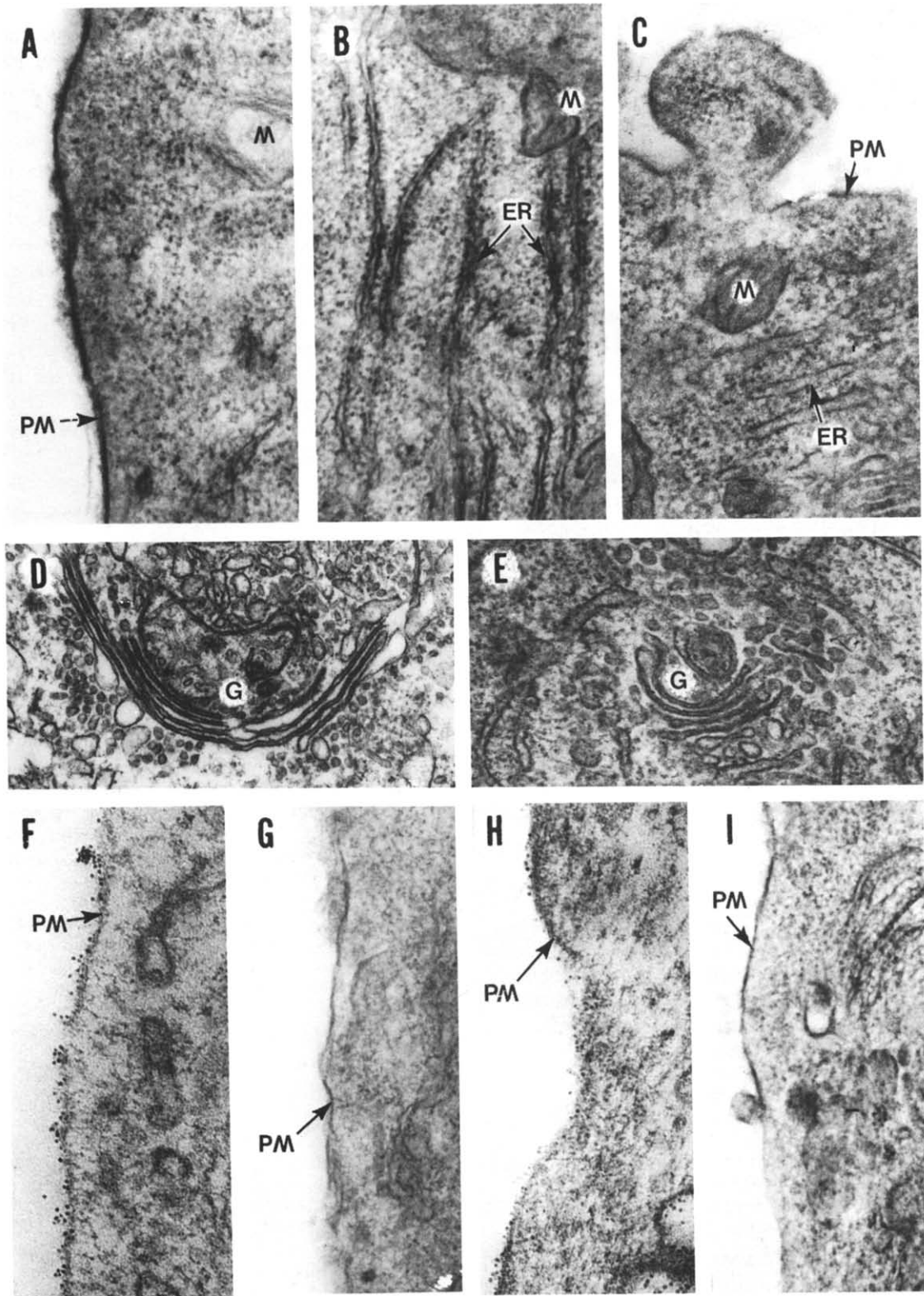


Fig.2

amount of purified human kidney transpeptidase was used (not shown). Further similarity between the lymphoid cell plasma membrane and the kidney enzymes was shown when the immunoprecipitate containing the tritiated enzyme from RPMI 8226 (labeled as in section 2; 2500 cpm/10 000 units) and the human kidney enzyme was subjected to SDS-polyacrylamide gel electrophoresis. Results of this experiment indicated that, like the kidney enzyme [6], the lymphoid cell enzyme is also composed of two glycoprotein subunits (mol. wt ~62 000 and 22 000, respectively) since radioactivity was found to co-migrate with the subunits of kidney enzyme. The heavy subunit region contained ~3-times as much radioactivity as the light subunit area.

Ultrastructural localization studies showed that transpeptidase activity in the cell line RPMI 8226 is seen in the plasma membranes as indicated by thicker and darker appearance of these membranes (fig.2A) (due to deposition of the lipophobic, osmophilic reaction product at the site of enzyme activity) as compared to the appearance of these membranes in the corresponding control (fig.2C). Longer incubation (2 h) of the cells with the cytochemical medium revealed enzyme activity in the membranes of the endoplasmic reticulum (fig.2B) and the Golgi (fig.2D). Similar localization of transpeptidase activity was seen in the cell line RPMI 1788 and in Con A-stimulated rat spleen cells (not shown). No activity was seen in mitochondria and nuclei.

Treatment of prefixed cells (RPMI 8226 and RPMI 1788) with anti-transpeptidase IgG-ferritin conjugate showed uniform binding of the conjugate to the plasma membranes (fig.2F and 2H, respectively). The greater labeling seen in the cell line RPMI 8226 as compared to that in RPMI 1788 is consistent with the higher transpeptidase activity in the former cell line. Since the ferritin conjugate does not permeate the cell membrane, intracellular membranes were not labeled under these conditions.

4. Discussion

Ultrastructural localization of γ -glutamyl transpeptidase activity and antibody inhibition studies demonstrate that the lymphoid cell plasma membrane is the primary site of the enzyme in these cells. Reaction with ferritin-antibody conjugate shows that the enzyme is uniformly distributed over the external sur-

face of the plasma membrane. Extended incubation of the cells in the cytochemical medium revealed activity on the membranes of the endoplasmic reticulum and the Golgi. The cytochemical method permits qualitative assessment regarding the relative activities in the intracellular and plasma membranes and is in accord with studies using impermeable substrate to compare the activities of intact cells and cell sonicates which showed that most of the cellular activity is indeed present on the cell surface [5]. Whether the enzyme on the intracellular membranes merely represents intermediate stages in the processing of this plasma membrane enzyme, shown to be a glycoprotein, or may in fact be significant in utilization of intracellular glutathione remains to be evaluated. The plasma membrane enzyme is similar in several respects to the purified kidney enzyme.

Intracellular glutathione can be translocated out of the lymphoid cells [2,12]. Localization of the major transpeptidase activity on the lymphoid cell surface suggests that the enzyme can interact with glutathione that is translocated to the cell surface as well as extracellular (plasma) glutathione. Also, the ease with which the enzyme can be localized and assayed, and the marked variation in the activity of cells derived from patients with various neoplastic diseases, may serve to make this enzyme a useful marker in studies relating to differentiation and neoplasia [5].

Acknowledgements

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